



Study of bacterial mastitis in dairy cattle of Pokhara Valley, Kaski Nepal

Ganga Sagar Bhattarai* and Sumit Gayek

Department of Microbiology, Prithvi Narayan Campus, Tribhuvan University, Kaski, Nepal
gbhattarai@pncampus.edu.np

Available online at: www.isca.in, www.isca.me

Received 12th August 2023, revised 7th September 2023, accepted 19th November 2023

Abstract

Mastitis is the most common disease in dairy cattle, and it has well-documented negative impacts on animal well-being and cattle farm profitability. It has become a major cause of milk loss from dairy farms as well as farmer economic losses. The goal of this study is to identify the bacteria that cause mastitis and their antibiogram profile. Three distinct bacteria were recovered from 145 milk samples. Cows (51%), and buffalo (49%), were among them. A total of 145 milk samples were tested for California mastitis (CMT) and 76 (52.41%) were processed. Bacterial growth was identified in just 68 (89.47%) of the 76 milk samples tested. Furthermore, 75% of the 68 mastitis positive milk samples contained *S. aureus*, 22% *S. epidermidis*, and 3% *Streptococcus*. Amikacin was highly effective against *S. epidermidis* and *S. aureus*, with sensitivity levels of 93.33% and 90.19%, respectively. Amikacin was 100% sensitive to *Streptococcus*, but Enrofloxacin was 50% sensitive to *Streptococcus*. *Staphylococcus aureus* showed 3 MDR with 3.92% and 1.96%.

Keywords: CMT, Mastitis, Antibiogram, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp.

Introduction

In developing countries, however, epidemiological and bacteriological studies of cattle mastitis in Nepal have not been completed. Mastitis is an inflammation of the mammary gland that typically develops in response to intra-mammary bacterial infection, but can also occur in response to intra-mammary mycoplasma, fungal, or algal infections. Mechanical, thermal, and chemical stress all predisposes the gland to intra-mammary infection (IMI). Mastitis is caused by the interaction of host, agent, and environmental variables. Milk is thought to be an ideal medium for the growth of many bacteria. Milk can be contaminated with microorganisms from milking staff and milking instruments during the milking process¹.

Mastitis is typically associated with physical, chemical, and bacterial abnormalities in milk, as well as pathological alterations in glandular tissue. It is the most expensive disease of dairy cattle, resulting in decreased potential production, quality and quantity losses. Mastitis is typically associated with physical, chemical, and bacterial abnormalities in milk, as well as pathological alterations in glandular tissue. It is the most expensive disease of dairy cattle, resulting in reduced potential output, losses in milk quality and quantity, losses due to rejected milk, early culling, veterinary costs, and labor costs².

Mastitis in cattle is divided into two types: clinical mastitis and subclinical mastitis. Clinical mastitis is easily identified by common symptoms such as flakes in milk and udder edema. Sub-clinical mastitis is frequently overlooked, yet it is more common than clinical mastitis, reduces output, and can readily progress to clinical mastitis under bad conditions.

Identifying and controlling subclinical mastitis is therefore critical among farmers. The average milk yield drop owing to clinical and subclinical mastitis was calculated to be 50 and 17.5%, respectively³.

Staphylococcal mastitis is one of the most prevalent infections associated with dairy animals. It typically manifests in a subclinical form and, if left untreated, can progress to clinical mastitis, resulting in significant financial losses for dairy owners. *Staphylococcus aureus* infections are difficult to control and have been linked to subclinical, clinical, and chronic mastitis, with treatment efforts typically failing. *Staphylococcus aureus* is an opportunistic organism that can cause a variety of diseases ranging from minor wound infections or food poisoning to bacteremia and other systemic infections⁴.

Dairy farming is now regarded as one of the most successful strategies of poverty alleviation, particularly in hilly areas where the majority of farmers are either resource poor or lack land. Due to rising demand and rising milk prices, it is becoming a successful enterprise for resource-poor farmers in the hills. Several studies, however, have demonstrated that clinical mastitis reduces milk supply. It can transmit significant zoonotic illnesses such as tuberculosis, leptospirosis, brucellosis, streptococcal sore throat, and gastroenteritis⁵.

Mastitis has become a major danger to breastfeeding animals' output. Mastitis is one of the most common dairy cow diseases in Nepal, as it is in other developing countries. Dairy animals are important for rural livelihoods in Nepal, but they frequently suffer from mastitis, a production disease that causes economic

losses to farmers, challenges to the dairy processing industry, and potential health hazards to consumers⁶.

It is also critical to investigate the sensitivity pattern of various bacteria isolated from mastitis cows in various geographical zones of the country in order to develop appropriate therapeutic methods using appropriate antibiotics⁷.

Given the foregoing, this study was conducted with the goals of isolating and identifying bacterial populations from California Mastitis Test (CMT) positive milk samples from livestock commercial farms, as well as designing their antibiogram profile. The identification of mastitis-causing pathogens and the results of antibiotic resistance testing on isolated bacteria are important prerequisites for the implementation of effective cattle mastitis control.

This information is required not only to treat and control mastitis, but also to support public health concerns.

Methodology

From January to March 2022, a descriptive cross-sectional investigation was carried out in the microbiology division of the Veterinary Laboratory in Pokhara. The aseptic approach was used to obtain 145 milk samples. Each donor was asked to complete a Questionnaire form and collect milk in a sterile glass bottle labeled with the appropriate details. Only CMT-positive milk samples were packaged in an ice chest and transported to the Microbiology laboratory for additional processing and analysis.

The morphology and features of colonies were observed. The modified Kirby-Bauer disc diffusion method was used to screen all isolates for antibiotic susceptibility.

Results and discussion

145 milk samples from various cattle ranches were gathered, processed, and examined in the Veterinary Laboratory in Pokhara. Of the 74 cows, 34 (23%) were Jersey and 40 (28%) were Holstein. Also, 71 (49%) of the Buffalo were Murrah in total.

Determination of milk sample by California mastitis test (CMT): CMT was positive in 76 (52.41%) of the samples and negative in 69 (47.58%).

Growth Pattern of microorganisms in the milk sample: Bacterial growth was seen in Jersey 10 (76.92%) and Holstein 18 (90%). Murrah 40 had the highest growth rate (93.02%).

Distribution of isolated microorganism from the milk sample: A total growth of 68 microorganisms from isolated bacterial species were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus* spp, where *Staphylococcus aureus* showed majority growth among isolated bacteria. Out of 51 *Staphylococcus aureus*, breed of buffalo (Murrah) showed highest isolates which is 28(54.90%) followed by jersey 9 (17.64%). Similarly, out of 15 *Staphylococcus epidermidis*, Murrah 11 (73.33%) showed highest isolates whereas Holstein showed lowest 4 (26.66%).

Table-1: Determination of milk sample by California mastitis test (CMT).

| CMT Test | Cow | | Buffalo | Total |
|----------|-------------|-------------|-------------|-------------|
| | Jersey | Holstein | Murrah | |
| Positive | 13 (8.96%) | 20 (13.79%) | 43 (29.65%) | 76 (52.41%) |
| Negative | 21 (14.48%) | 20 (13.79%) | 28 (19.31%) | 69 (47.58%) |
| Total | 34 (23.44%) | 40 (27.58%) | 71 (48.96%) | 145 |

Table-2: Growth pattern of microorganisms in the milk sample.

| Sample | Variety | Growth | No growth | Total |
|--------------|----------|-------------|------------|-------------|
| Cow Milk | Jersey | 10 (76.92%) | 3 (23.07%) | 13 (17.10%) |
| | Holstein | 18 (90%) | 2 (10%) | 20 (26.31%) |
| Buffalo Milk | Murrah | 40 (93.02%) | 3 (6.97%) | 43 (56.57%) |
| Total | | 68 (89.47%) | 8 (10.52%) | 76 |

Antibiotic susceptibility pattern of the isolated organism: Staphylococcus aureus made up 51 of the 68 isolated organisms, followed by Staphylococcus epidermidis (15), and Streptococcus spp.

Among 51 isolated Staphylococcus aureus, Amikacin 46 (90.19%) and Ceftriaxone 28 (54.90%) demonstrated the highest levels of sensitivity in S. aureus. Enrofloxacin 11 (21.26%) and amikacin 1 (1.96%) were both highly resistant to S. aureus.

Similar to the other Staphylococcus epidermidis isolates, S epidermidis exhibited the maximum sensitivity to Amikacin 14 (93.33%), which was followed by ceftriaxone 6 (40%) in the group of 15 isolates. Tetracycline and enrofloxacin both displayed the highest levels of resistance 4 (26.66%).

Last but not least, no Streptococcus spp. resistance was discovered among the 5 Antibiotics that were recovered from 2 separate Streptococcus spp.

Discussion: The study included 145 bovine milk samples obtained from several commercial cattle farms in the Pokhara valley, with the goal of isolating mastitis-causing bacteria in the

milk samples. Out of 145 cattle milk samples, 76 (52.41%) were CMT positive, while 69 (47.58%) were CMT negative. CMT positive samples were taken to the Pokhara Veterinary Laboratory to be processed and analyzed. Positive growth of an organism was observed in 68 (89.47%) of Positive CMT, while 8 (10.52%) CMT positive did not display their growth pattern on selective media. 68 organisms were isolated from 76 samples. From the CMT positive sample, three different kinds of bacteria were identified. Whereas 51 Staphylococcus aureus, 15 Staphylococcus epidermidis, and 2 Streptococcus spp. were recovered from Murrah 28 (54.90%), Staphylococcus epidermidis were isolated from Murrah 11 (73.33%). The antibiotic susceptibility of the isolated organism was evaluated using the Kirby-Bauer disk diffusion method with four distinct antibiotic groups.

145 milk samples were collected, were 74 cows and 71 buffaloes. Besides, cows came in two varieties. They were Jersey 34 and Holstein 40. The livestock distributions are shown in Table-4. All of the cattle chosen were lactating less than a month ago. Cows are the most numerous among all cattle. Bovine mastitis is a severe concern around the world due to the economic loss in dairy farms, where the prevalence ranges from 48.62% to 86.2% in cows⁸.

Table-3: Distribution of isolated microorganism from milk sample.

| Sample | Variety | Staphylococcus aureus | Staphylococcus epidermidis | Streptococcus spp | Total |
|--------------|----------|-----------------------|----------------------------|-------------------|-------|
| Cow milk | Jersey | 9 (17.64%) | 0 | 1 (50%) | 10 |
| | Holstein | 14 (27.45%) | 4 (26.66%) | 0 | 18 |
| Buffalo Milk | Murrah | 28 (54.90%) | 11 (73.33%) | 1 (50%) | 40 |
| Total | | 51 | 15 | 2 | 68 |

Table-4: Antibiotic susceptibility pattern of isolated organism.

| Antibiotics | Staphylococcus aureus | | | Staphylococcus epidermidis | | | | Streptococcus | |
|---------------|-----------------------|-----------|-----------|----------------------------|----------|----------|--------|---------------|-------|
| | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Ciprofloxacin | 34(66.66) | 10(19.60) | 7(13.72) | 12(80) | 3(20) | 0 | 2(100) | 0 | 0 |
| Ceftriaxone | 28(54.90) | 19(37.25) | 4(7.84) | 6(40) | 7(46.66) | 2(13.33) | 2(100) | 0 | 0 |
| Tetracycline | 44(86.27) | 1(1.96) | 6(11.76) | 8(53.33) | 3(20) | 4(26.66) | 2(100) | 0 | 0 |
| Enrofloxacin | 36(70.58) | 4(7.84) | 11(21.56) | 7(46.66) | 4(26.66) | 4(26.66) | 1(50) | 1(50) | 0 |
| Amikacin | 46(90.19) | 4(7.84) | 1(1.96) | 14(93.33) | 1(6.66) | 0 | 2(100) | 0 | 0 |
| Gentamicin | 45(88.23) | 4(7.84) | 2(3.92) | 13(86.66) | 2(13.33) | 0 | 2(100) | 0 | 0 |

The screening of mastitis can be done by California Mastitis Test (CMT) in cattle farm. CMT positive sample were 76 out of total sample 145. The field wise screening of this test is also called sub-clinical mastitis. The preliminary test done by CMT in positive milk sample were collected for further analysis and processing for causative agent. Our results showed 76 (52.14%) CMT positive sample which was least finding 56.0% to the study⁹. This study represents prevalence of the mastitis is higher in buffalo 43 (29.65%), the highest CMT positive sample result was 52.4%, which was similar to the findings. This finding demonstrated that the scenario in north Ethiopia where cow breed rearing is higher in large scale dairy farms. In contrast, 38.07% prevalence is found in buffalo, which is the least similar to our findings. A big number of gram positive group of organisms were identified from the cow milk sample from the dairy farm in the current study. Three types of bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus spp.*, were isolated for this investigation, which is consistent with the results. From the cattle milk sample, we identified 75% *Staphylococcus aureus*, which is comparable to the results, which isolated 73.2% of an organism¹⁰. Our findings varied from 65.9 to 97.7%, with a 72.7% similarity percentage to Gram positive bacteria discovered. In a study on mastitis milk, the pathogenic species of *Staphylococcus aureus* were the ones that were most frequently isolated from the milk sample. According to a study, *Staphylococcus aureus* prevalence was 4.8%, which is quite low when compared to our findings. Although one study found 45% *Staphylococcus aureus* in buffalo milk, we found 54.90% *Staphylococcus aureus* in buffalo (murrh) milk, the lowest of any study. The buffalo had the highest quantity of *Staphylococcus aureus* (54.90%) compared to the cow, which is opposite and least to the findings¹¹.

In this investigation, 22% *Staphylococcus epidermis* was isolated from the sample, which is comparable to the findings of 18.20%. The percentage of *Staphylococcus epidermidis* isolated was 11.5%. However, *Staphylococcus epidermidis* was identified from cattle milk samples at a rate of 28%. This scenario depicts the dispersion of *Staphylococcus epidermidis* in several ecological niches, with the distribution influenced by environmental factors and others¹². Mastitis in cattle is connected with climatic conditions; the most number and species of bacteria were recovered in the summer season, with the most prevalent pathogen in mastitis positive milk samples. Similarly, 17% *Staphylococcus epidermidis* and 5.62% *Streptococcus spp* were recovered from a buffalo mastitis positive milk sample¹³. We isolated the same number of Bactria from the cow and buffalo. Buffalo milk contains 23% *Streptococcus spp*. It could be attributed to seasonal variations, proper farming methods, and the teat dipping process.

Antibiotics are a miraculous medicinal discovery that can cure bacterial illnesses. Our results showed that *S. aureus*, *S. epidermidis*, and *Streptococcus spp*. had the highest sensitivity to Amikacin (90.19%) and the lowest result (70%). The highest

level of resistance to Enrofloxacin was 21.56%, which was reduced to 69%¹⁴. *Staphylococcus epidermidis* is 93.33% susceptible to amikacin, both Tetracyclines and Enrofloxacin were resistance 26.66% in our work but other study gets 86.25 sensitive towards Enrofloxacin which is opposite as compare to our result. Less resistance means highest sensitivity of antibiotics, similar results of 25% were found. In our study, two *Streptococcus* species were identified, and all of them were 100% sensitive to antibiotics, with the exception of Enrofloxacin 50%, which was 53% identical to the findings¹⁵.

51 isolated *Staphylococcus aureus* were tested for multidrug resistance, and the resistance to three medications was 3.92%, *Staphylococcus aureus* was primarily resistant to penicillin and its associated medication, but we did not utilize any antibiotics that were penicillin-related. In addition, research should be conducted to detect resistance in *Staphylococcus aureus*. Our results were lower than 24.50% in the preceding debate¹⁶. Antibiotic resistance, on the other hand, is spreading like wildfire. Policymakers and the DDA (Department of Drug Administration) should conduct surveillance to discover this condition both globally and in Nepal.

Conclusion

The investigation was carried out to identify the mastitis-causing bacteria and their antibiogram profile in cattle in the Pokhara valley. A total of 145 cow milk samples were collected. 76 (52.41%) of the 145 milk samples were positive. CMT positive samples were taken to the veterinary laboratory in Pokhara and cultured on selected medium for the isolation of mastitis-causing bacteria. Biochemical tests were used to identify various bacteria (89.47%). The most common bacteria isolated were *Staphylococcus aureus* and *Streptococcus*. Amikacin was shown to be the most sensitive (93.33%) in *S. epidermidis*, followed by Ceftriaxone (40%), and resistance was discovered to be Enrofloxacin (21.56%) in *S. epidermidis*. *Streptococcus* was discovered to be antibiotic sensitive among all bacteria. MDR was shown to be (3.92%) resistant to three medicines.

Recommendation: The study suggests that, the farm sanitation, farm hygiene and other parameters should be monitored for identifying source of possible contamination and proper bioremediation techniques should be carried out to improve the farm and milk quality. Burden of antibiotic resistance is spread like a wildfire. Therefore, the policy makers and DDA (Department of Drug Administration) should surveillance to detect this situation world-wide and also in Nepal.

Acknowledgement

The authors are thankful to the authority of Veterinary Laboratory, Pokhara, and giving permission to carry out the research on Mastitis. Special thanks go to Dr. Ganesh K.C. and also owners of different cattle farms to perform the research.

References

1. Rehman, M. U., Rashid, M., Sheikh, J. A., & Bhat, M. A. (2014). Molecular epidemiology and antibiotic resistance pattern of enteropathogenic *Escherichia coli* isolated from bovines and their handlers in Jammu, India. *Journal of Advanced Veterinary and Animal Research*, 1(4), 177-181.
2. Bangar, Y. C., Singh, B., Dohare, A. K., & Verma, M. R. (2015). A systematic review and meta-analysis of prevalence of subclinical mastitis in dairy cows in India. *Tropical animal health and production*, 47, 291-297.
3. Bhattarai, A., Kaphle, K., & Adhikari, P. (2020). A Review on Bovine Sub-Clinical Mastitis in Nepal: Sustainable Management Strategy. *International Journal of Food Science and Agriculture*, 4(1), 80-89.
4. Straub L, Stegger M, Akpaka P E, Alabi A, Breurec S, Coombs G, Egyir B, Larsen A R, Laurent F, Monecke S, Peters G, Skov R, Strommenger B, Vandenesch F, Schaumburg F and Mellmann, A (2017). Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. *Proceedings of the National Academy of Sciences of the United States of America*, 114(49), E10596–E10604.
5. Khan, A. Z., & Muhammad, G. (2005). Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. *Pakistan Veterinary Journal*, 25(1), 9-12.
6. Sah, K., Karki, P., Shrestha, R. D., Sigdel, A., Adesogan, A. T., & Dahl, G. E. (2020). MILK Symposium review: Improving control of mastitis in dairy animals in Nepal. *Journal of dairy science*, 103(11), 9740-9747.
7. Haque, M. E., Islam, M. A., Akter, S., & Saha, S. (2014). Identification, molecular detection and antibiogram profile of Bacteria isolated from California mastitis test positive milk samples of crossbred cows of Satkhira district in Bangladesh. *GSTF Journal of Veterinary Science (JVet)*, 1(1).
8. Yang, W. T., Ke, C. Y., Wu, W. T., Lee, R. P., & Tseng, Y. H. (2019). Effective treatment of bovine mastitis with intramammary infusion of *Angelica dahurica* and *Rheum officinale* extracts. *Evidence-Based Complementary and Alternative Medicine*, 2019.
9. Gitau, G. K., Bundi, R. M., Mulei, C. M., & Vanleeuwen, J. (2014). Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity. *Journal of the South African Veterinary Association*, 85(1), 1-8.
10. Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC veterinary research*, 12(1), 1-11.
11. Khan, J. M., Rasool, M. H., Arshad, M., Rahman, S. U., Tahir, M. F., Aslam, B., ... & Ghani, M. (2013). Comparative evaluation of Leukotoxic activities of indigenous *Staphylococcus aureus* isolates from subclinical and clinical mastitic milk samples of buffalo and cattle. *The Open Veterinary Science Journal*, 7(1).
12. Mehmeti, I., Behluli, B., Mestani, M., Ademi, A., Nes, I., & Diep, D. B. (2016). Antimicrobial resistance levels amongst staphylococci isolated from clinical cases of bovine mastitis in Kosovo.
13. Vásquez-García, A., Silva, T. D. S., Almeida-Queiroz, S. R. D., Godoy, S. H., Fernandes, A. M., Sousa, R. L., & Franzolin, R. (2017). Species identification and antimicrobial susceptibility profile of bacteria causing subclinical mastitis in buffalo. *Pesquisa Veterinária Brasileira*, 37, 447-452.
14. Hermana, N. S. P., Nilasari, Z., Yuniarti, E., Riyanto, A., Indrawati, A., & Pasaribu, F. H. (2020). Antibiotic resistance profile of *Staphylococcus aureus*, *Streptococcus* spp. and *Klebsiella* spp. isolated from chicken farm in Bogor, Sukabumi, and Cianjur, West Java. In *Journal of Physics: Conference Series*, Vol. 1430, No. 1, p. 012021. IOP Publishing.
15. Abd El-Razik, K. A., Arafa, A. A., Hedia, R. H., & Ibrahim, E. S. (2017). Tetracycline resistance phenotypes and genotypes of coagulase-negative staphylococcal isolates from bubaline mastitis in Egypt. *Veterinary world*, 10(6), 702.
16. Kayalvizhi, V., & Usha, A. (2014). Microbial and physico-chemical changes in tomato juice subjected to pulsed electric field treatment. *African Journal of Microbiology Research*, 8(37), 3382-3391.